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Original Article **Open Access**

Comparison of serum and synovial fluid markers of Herpes simplex virus and *Helicobacter pylori* infection between rheumatoid arthritis and osteoarthritis patients: A Retrospective Case-Control Study

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Recently, several infectious agents including Epstein-Barr virus and Escherichia coli have been suggested as possible contributing factors to the pathogenesis of rheumatoid arthritis (RA). This study was designed to compare serum and synovial fluid markers of herpes simplex virus (HSV) and *Helicobacter pylori* of RA and osteoarthritis (OA) patients.

This comparative study was conducted on two hundred OA and RA patients who referred to the Rheumatic Diseases Research Center (RDRC) affiliated with Mashhad University of Medical Sciences, Mashhad, Iran, from March 2015 to 2016. Synovial fluid was obtained from all individuals. Two years later, participants attended a follow-up session to collect blood samples for serum markers of these two infectious agents.

Twenty-five patients (96.15%) in the RA group and 23 individuals (92%) in the OA group had positive serum IgG antibodies for HSV. As for *Helicobacter pylori*, 13 individuals (50%) in RA and 12 individuals (48%) had positive serum IgG antibodies (*p value* = 0.66). In addition, 9 (34.6%) and 8 (30.8%) in the RA group and 10 (40%) and 3 (12%) in the OA group had positive serum IgA and IgM antibodies for *Helicobacter pylori*, respectively (*p value* = 0.89 and *p value* = 0.13, respectively). Collected fluid samples were negative for both *Helicobacter pylori* and HSV1 and 2 DNA particles in all individuals.

Based on the results of the current study, there is no difference between RA and OA patients in terms of Herpes simplex virus and *Helicobacter pylori* infection.

Keywords: Helicobacter pylori, Herpes simplex virus, Osteoarthritis, Rheumatoid arthritis, Synovial fluid

Introduction

Rheumatoid arthritis (RA) is one of the most common inflammatory conditions of unknown etiology, characterized by symmetrical peripheral polyarthritis [1, 2].

Several infectious agents have been suggested as potential contributing factors in the development of RA, including both bacterial (i.e. *Helicobacter pylori* and Mycoplasma pneumonia) and viral (i.e. Cytomegalovirus, Epstein-Barr virus, Parvovirus B19, and retroviruses) agents. Immune complexes formed as a result of immune reactions to infectious agents lead to an induction of RA-related factors. One of these is the rheumatoid factor (RF), which has a high affinity to the Fc region of immunoglobulins [3].

Additionally, infection results in an increased permeability of the synovial membrane to leukocytes, which leads to their accumulation and an increased expression of adhesion molecules (i.e. integrins, selectins, laminins, and fibronectins) and immunoglobulins. Gene expression of inflammatory cytokines is also increased, leading to neoangiogenesis in tissue [4, 5]. These changes, together with activation of regional fibroblasts, cause the formation of inflammatory structural changes similar to those seen in RA [6].

Several epidemiologic studies have shown an association between periodontal diseases and RA [7-9]. In addition, Porphyromonas gingivalis and Escherichia coli, both of which are involved in infectious periodontal diseases, also play roles in the production of autoantibodies, citrullination of peptides, and synovial inflammation [10].

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Viral agents, alone or together with bacterial pathogens, can either directly or indirectly through induction of autoimmune reactions damage joints. Several previous studies have evaluated the association between *H. pylori* and the development of RA, and some of them established an association between *H. pylori* infection and elevated disease markers of RA [11]. Nonetheless, a cohort study with a relatively large sample size found no difference in the risk of acquiring RA between *H. pylori* positive and negative patients in a follow-up period of 8 years [12].

Due to the controversial findings in the literature on the relationship between *H. pylori* infection and autoimmune disease, this study aimed to compare the serum markers of infection with Herpes simplex virus 1 (HSV-1) and *Helicobacter pylori* between RA patients and OA patients.

Materials and Methods

This comparative study was conducted on OA and RA patients who referred to the Rheumatic Diseases Research Center (RDRC) affiliated with Mashhad University of Medical Sciences, Mashhad, Iran, from March 2015 to 2016.

Inclusion and Exclusion Criteria

The inclusion criteria were age over 18 years and a diagnosis of grade 3 or 4 OA based on the Kellgren and Lawrence criteria or RA established by a rheumatology subspecialist based on the American College of Rheumatology criteria, 2010. Following inclusion, patients suspected of having septic arthritis, had crystals on microscopic evaluation of their synovial fluid, or had consumed any form of antibiotic during the two weeks prior to sampling as well as patients with no available medical records or a history of herpes labialis during the 3 months prior to sampling were excluded.

Study Design

This study was performed on all OA and RA patients who referred to RDRC from March 2015 to March 2016. In general, 200 patients (100 established RA cases and 100 OA patients) were randomly selected to participate in this study. The data was gathered by a researcher-made questionnaire covering demographic information which was completed for each case separately. Synovial fluid aspiration (2-5 milliliters) was performed by a rheumatology subspecialist using sterile aspiration needles and sterile tubes with no additives, and the samples were stored at -20 °C under appropriate laboratory conditions to be analyzed later.

According to the results of the evaluation of medication consumption, the RA patients consumed prednisolone (83%), methotrexate (73%), hydroxychloroquine (33%), sulphasalizine (26%), and biologic medicines (9%) in descending order. Considering the disease duration, the results revealed no significant difference between the two groups in this regard (*p value* = 0.07).

Arthrosynthesis had been performed in RA and OA patients due to knee effusion in 2015 to 2016. These patients required synovial fluid drainage as part of their treatment process. Drainage of joint fluid was performed by a rheumatologist considering the principles of aseptic technique.

All synovial fluid samples were evaluated for crystalopathies and infections, and suspicious cases were excluded from the study. The remaining samples were centrifuged at 12000 g for 30 minutes and stored at -20 °C. All laboratory operators were blinded to clinical diagnoses. In 2018, 26 individuals from the RA group and 25 individuals from the OA group attended a follow-up session during which venous blood samples (50-10 milliliters) were taken by a laboratory operator using sterile needles. The obtained samples were centrifuged at 1200 g for 10-15 minutes and stored at -20 °C to be later analyzed for serum markers of infection.

Laboratory analyses

Real-time PCR (RT-PCR) for HSV1 and 2 and *H. pylori*'s DNA was applied to remove the viscosity of synovial fluid. The samples were washed by phosphate buffered saline (PBS), and then the DNA was extracted using a Genetbio® commercial kit (South Korea) according to the manufacturer's instructions. After DNA extraction, RT-PCR was performed using a Fanavari Novin kit (Tehran, Iran) according to the manufacturer protocol. The master mix was inserted in designated microtubes. For PCR cycling, 30 µl of Master Mix was added to 10 µl of the isolated DNA. A thermocycler was used to amplify the DNA content of the samples in PCR cycling conditions: denaturation phase (at 98 °C for 15 seconds), annealing phase (at 63 °C for 60 seconds), and extension phase (at 72 °C for 120 seconds). This cycle was repeated 60 times. Following that, Agarose gel electrophoresis was used to detect and quantify the DNA contents. Finally, the data was analyzed using Rotor-Gene 6000 software [13].

Enzyme-linked immunosorbent assay (ELISA) for serum markers of HSV1 and 2 and *H. pylori* infection was performed using a Pishtaz Teb Zaman Diagnostics (Tehran, Iran) ELISA kit. 10 µl of serum sample was mixed with 1 milliliter of diluting solution, and 100 µl of the mixture was added to each well on the ELISA plate. Standard, control, and sample dilutions were added to their designated wells. Following that, the plate was incubated at room temperature (22-28 °C) for 30 minutes. After the incubation period, each well was emptied and then washed using detergent solution five times. Immediately following that, 100 µl of antibody-enzyme conjugate. The ELISA plate was then incubated at room temperature (22-28 °C) for an additional 30 minutes. Then the washing process was performed five more times. 100 µl of Colorimetric substrate was added to each well and the palate underwent 15 minutes of incubation at room temperature (22-28 °C) in a dark environment. Immediately after that, 100 µl of stop solution was added to each well, and the resulted mixture was analyzed using the ELISA reader machine at the wavelength of 450 nanometers and using a reference wavelength of 630 nanometers. Finally, the standard curve was used for determining the concentration of the provided samples.

Statistical Analysis

Statistical analyses were carried out using IBM-SPSS software version 21. The normality of the data was assessed using the Kolmogorov-Smirnov test. After the normality of data was calculated, the t-test or its nonparametric equivalent test (i.e. Mann-Whitney U test) was employed to analyze the variables. Moreover, the chi-square and Fisher exact test were used to analyze the data. A *p value* less than 0.05 was considered statistically significant.

Results

This study enrolled 200 patients, and the majority of them were female (n = 161, 80.5%).RA and OA groups included 77 (77%) and 23 (23%) and 84 (84%) and 16 (16%) females and males, respectively, which showed no significant difference between the two groups in terms of gender (*p value* = 0.21). Table 1 summarizes the mean ages and durations of illness of the two groups. Regarding age, 91% of the OA patients were more than 50 years old, and the patients in the OA group were significantly older than those in the RA group (*p value* < 0.05).

Table 1. The mean of age and duration of illness between two groups

Variables	RA group		OA group		*p value*
	Mean	SD	Mean	SD	
Age (years)	51.91	14.92	61.37	9.85	<0.005
Duration of illness (years)	8.41	6.28	7.07	3.73	0.07

Serological data of RA patients revealed that the mean erythrocyte sedimentation rate (ESR) and anti-CCP were 34.18 ± 2.57 mm/h and 122.10 ± 1.59 IU/ml, respectively. Furthermore, the anti-CCP parameter (82%) and ESR (58%) rates were higher than normal, and RF was positive in 77% of patients in the RA group.

The PCR analysis of joint fluid in both groups showed that all samples were negative for herpes virus DNA; however, two of the synovial fluid samples were found to be positive for *Helicobacter pylori*.

Table 2 tabulates the mean concentration values of HSV IgG in the two groups. According to the results, there is no statistically significant difference between the RA and OA groups (*p value* = 0.66), RF-negative and -positive groups (*p value* = 0.21), positive and negative anti-CCP groups (*p value* = 0.2), or positive and negative ESR groups (*p value* = 0.13) in terms of HSV IgG level. Furthermore, female RA patients and their OA counterparts (*p value* = 0.67) showed no statistically significant difference with male RA patients and their OA counterparts (*p value* = 0.82).

Table 2. The rate of IgG Herpes virus in patients

Variable			Mean±SD	t-test	*P value*
Groups	RA		176.83±86.88	-0.443	0.660
	OA		187.83±86.27		
Gender	Men	RA	187.44±70.95	-0.231	0.825
		OA	197.73±32.85		
	Female	RA	174.30±91.62	-0.426	0.673
		OA	180.30±91.58		
RF	Positive		166.36±83.22	1.275	0.215
	Negative		220.80±97.75		
ESR	Positive		202.69±67.45	-1.561	0.132
	Negative		150.97±98.62		
ANTI-CCP	Positive		166.15±83.39	1.302	0.205
	Negative		221.69±96.40		

Other results showed that the positive *H. pylori*'s serum IgG condition had no statistically significant difference between the two groups (*p value* = 0.82). No statistically significant difference was observed between the two groups in terms of *H. pylori*'s serum IgA (*p value* = 0.89) or serum IgM (*p value* = 0.13). Table 3 presents the two groups in terms of positive *H. pylori*'s serum IgG, IgA, and IgM.

The *Helicobacter pylori* serum IgG levels were estimated at 72.7% and 27.3% in the RF-positive and -negative patients, respectively. In addition, there was no statistically significant difference in IgG levels between the two groups (*p value* = 0.58).

Table 3. Comparison between two groups in terms of positive *H. pylori*'s serum IgG, IgA, and IgM

Variables	RA group	OA group	*P value*
Serum *H. pylori* IgG (IU/ml)	50%	48%	0.82
Serum *H. pylori* IgA (IU/ml)	34.6%	40%	0.890
Serum *H. pylori* IgM (IU/ml)	30.8%	12%	0.130

Discussion

Based on the PCR analysis of joint fluid in the RA and OA groups, all samples were negative for herpes virus DNA; however, two of the synovial fluid samples were found to be positive for *Helicobacter pylori*. The current study shows that there is no significant difference between RA and OA patients for infection with HSV1, 2 or *H. pylori*.

Due to the lack of basal membrane in the synovium or its damage in patients with rheumatoid arthritis and osteoarthritis, these people are prone to infection with HSV [14]. On the other hand, the proliferation of microbes affects the immune system response and the release of inflammatory mediators, including interleukin-3 and TNF-alpha, leading to cartilage, synovitis, and joint destruction [15]. In a large cohort study by Bartels et al., the researchers evaluated a total of 56,000 patients for *H. pylori* using the urea breath test (UBT); these patients also underwent a follow-up period of 8 years. Their results indicated that there was no significant difference in terms of incidence of new RA cases between *H. pylori* positive and negative patients during the course of the follow-up period [12]. Saad et al. demonstrated no significant clinical or laboratory differences between *Helicobacter pylori* positive and negative patients in terms of rheumatoid [16]. Similarly, no difference was reported between RA and OA patients with *Helicobacter pylori* positive and negative findings regarding the clinical and laboratory results, which is consistent with the results of the present study.

The prevalence rate of *Helicobacter pylori* in autoimmune diseases was not remarkable, as it was found that *Helicobacter pylori* infection increased with age [17]. In this study, the majority of OA patients were more than 50 years old, and data analysis showed that the patients in the OA group were older than those in the RA group.

The current study also showed that the presence of HSV DNA in the synovial space in one-third of the patients with RA could be due to the patient's temporary exposure to HSV. Additionally, the presence of HSV DNA in joint fluid indicates that the DNA of this virus has entered the space and joint fluid through white blood cells from the systemic bloodstream. HSV DNA was negative in the joint despite the positive blood level, which may be due to the distance between sampling, the consumption of antibiotics, or other detectable variables.

The probable effect of HSV infection as an independent factor on the development of immune system disorders, inflammation, and autoimmunity was not remarkable in the current study. Generally, the PCR of HSV types 1 and 2 was not positive in any of the joint fluid samples of the patients with RA and OA in the current study.

Similarly, no trace of the herpes virus was reported in joint fluid or serum samples of the OA patients in the above-mentioned study; therefore, it can be surmised that both studies were conducted in the same method.

The present study showed no difference between the RA and OA groups. Burgos et al. showed that IgG and IgM levels were above normal in 50% of RA patients. Furthermore, no significant difference between the level of antibody and PCR was reported in the analysis of IgG and IgM antibodies against HSV in serum and joint fluid [18]. Larionova et al. utilized serological methods to study IgG and IgM, and their results indicated that increased IgM anti-human herpes virus response in RA patients is related to HSV1/2 reactivation [19].

H. pylori infection as a stimulating and intensifying factor in autoimmune diseases was confirmed by Smyk et al. [20]. There is evidence on the cross reactivity between T-cells and the production of autoantibodies in autoimmune disease patients with *H. pylori* infection [21]. Furthermore, Kandil et al. studied idiopathic juvenile arthritis patients and found that compared with healthy children, patients with juvenile idiopathic arthritis had a 34.1% greater chance of being seropositive for *H. pylori* [22].

It is of paramount importance to mention that *H. pylori* can be an exacerbating factor for RA or related conditions. It is hypostasized that *H. pylori* infection leads to aggravated symptoms and inflammation in autoimmune diseases. For example, in a recent study performed on 100 RA patients by Ebrahimi et al., researchers found that *H.*

pylori seropositive RA patients had significantly higher serum levels of rheumatoid factor (RF), ESR, CRP, anti-cyclic citrullinated peptide (Anti-CCP), and anti-mutated citrullinated vimentin (Anti-MCV) than their seronegative counterparts. Moreover, they found that *H. pylori* seropositive RA patients who also had a positive cytotoxin-associated gene A protein (CagA) also scored significantly higher on disease activity score 28 (DAS28) and visual analogue scale (VAS) than their CagA negative counterparts [23]. Jones et al. estimated the presence of *H. pylori* infection to be 43-68% among RA patients, [24] which is consistent with the findings of the study performed by Ebrahimi et al. [23]. Zentilin et al. confirmed the results of the aforementioned study on the higher rate of ESR and CRP in RA patients with *H. pylori* infection in comparison to those who are not infected by *H. pylori* [25].

It should be noted that development of systemic rheumatic disease is not dependent exclusively on an infectious agent. Therefore, *H. pylori* infection may be only an underlying factor for the expression of particular genes leading to autoimmune diseases. This might be due to an interaction between the infectious agent and a cascade of host-specific factors [11].

According to the results of the current study, however, it is more probable that *Helicobacter pylori* has no obvious effect on RA and OA prevalence or incidence. Although the sufficiency of the follow-up length is debatable, the results show that infection with *Helicobacter pylori* is unlikely to be a risk factor for the development of RA, which is consistent with the results of the current study.

The key limitation of the current study was the lack of follow-up after the commencement of *H. pylori* eradication therapy to assess the clinical outcomes of RA patients who were seropositive for this agent. The other limitation of this study was the lack of adjusting the effects of age on the comparisons of the serum markers of infection with Herpes simplex virus 1 (HSV-1) and *Helicobacter pylori* between RA and OA patients. The authors suggest that future studies include a follow-up in long enough intervals after eradication therapy to evaluate how RA patients have responded when compared to their baseline.

Conclusion

Based on the results of the current study, there is no difference between RA and OA patients in terms of Herpes simplex virus and *Helicobacter pylori* infection. The results further suggest that because the difference in serum marker levels and OA patients is not significant and that the DNA particles of both agents are absent in the synovial fluid of RA patients, it is unlikely that either HSV1, 2, or *H. pylori* is a risk factor for the development of RA.

Declarations

Ethical Considerations

This study was approved by the Ethics Committee of Mashhad University of Medical Sciences, faculty of medicine which can be accessed using the code “IR.MUMS.sm.REC.1395.614”. All patients filled out an informed consent form and were free to leave at any point during the study. All of the collected data were kept confidential and the patients’ names were excluded from the data. None of the authors of this study had conflict of interest or financial ties to disclose.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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None.

Author Contributions

This work was carried out in collaboration among all authors. MS designed the study, YS performed the statistical analysis, HR performed Laboratory analyses, MKH wrote the protocol, and wrote the first draft of the manuscript. SS and MG managed the analyses of the study. HH and KH managed the literature searches. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

References

1. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. ***N Engl J Med*** 2011; 365(23):2205-19. doi:10.1056/NEJMra1004965.
2. Arvikar SL, Crowley JT, Sulka KB, Steere AC. Autoimmune arthritides, rheumatoid arthritis, psoriatic arthritis, or peripheral spondyloarthritis following Lyme disease. ***Arthritis Rheumatol*** 2017; 69(1):194-02. doi:10.1002/art.39866.
3. Scherer HU, van der Linden MP, Kurreeman FA, Stoeken-Rijsbergen G, le Cessie S, Huizinga TW. *et al*. Association of the 6q23 region with the rate of joint destruction in rheumatoid arthritis. ***Ann Rheum Dis*** 2010; 69(3):567-70. doi:10.1136/ard.2008.106161.
4. Sarithala VJ, Koroy P, Yagoda A.Variations in levels of intercellular adhesion molecule-1 with disease course, in rheumatoid arthritis patients. ***J Clin Dia Res*** 2018;

12(9):18-21. doi:10.7860/JCDR/2018/35302.12044.

5. Koroy P, Sarithala V, Yagoda A. Soluble molecules of immunoglobulins superfamily in rheumatoid arthritis. ***Med News North Caucasus*** 2017; 12(3):256-60. doi:10.14300/mnnc.2017.12092.

6. Scher JU, Littman DR, Abramson SB. Microbiome in inflammatory arthritis and human rheumatic diseases. ***Arthritis Rheumatol*** 2016; 68(1):35-45. doi:10.1002/art.39259.

7. Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. ***Int J Health Sci (Qassim)*** 2017; 11(2):72-80.

8. Fabri GM, Savioli C, Siqueira JT, Campos LM, Bonfá E, Silva CA. Periodontal disease in pediatric rheumatic diseases. ***Rev Bras Reumatol*** 2014; 54(4):311-17. doi:10.1016/j.rbr.2013.11.004.

9. Detert J, Pischon N, Burmester GR, Buttgereit F. The association between rheumatoid arthritis and periodontal disease. ***Arthritis Res Ther*** 2010; 12(5):218. doi:10.1186/ar3106.

10. Hitchon CA, Chandad F, Ferucci ED, Willemze A, Ioan-Facsinay A, van der Woude D. *et al*. Antibodies to Porphyromonas gingivalis are associated with anticitrullinated protein antibodies in patients with rheumatoid arthritis and their relatives. ***J Rheumatol*** 2010;37 (6):1105-12. doi:10.3899/jrheum.091323.

11. Jafarzadeh A, Nemati M, Rezayati MT ,Nabizadeh M, Ebrahimi M. Higher serum levels of rheumatoid factor and anti-nuclear antibodies in *Helicobacter pylori*-infected peptic ulcer patients. ***Oman Med J*** 2013; 28(4):264-69. doi:10.5001/omj.2013.74.

12. Bartels L, Pedersen A, Kristensen N, Jepsen P, Vilstrup H, Stengaard-Pedersen K. *et al*. *Helicobacter pylori* infection is not associated with rheumatoid arthritis. ***Scand J Rheumatol*** 2019; 48(1):24-31. doi:10.1080/03009742.2018.1464205.

13. Sahebari M, Heidari A, Rastin M, Hashemzadeh K. Real-time PCR of Mycoplasma pneumoniae in Synovial Fluid of Rheumatoid Arthritis and Osteoarthritis Patients. ***Ann Arthritis Clin Rheumatol*** 2018; 1(1).1005.

14. Lee EY, Hong S-J, Park Y-B, Park K-S, Choi C-B, Lee C-K, *et al*. Gastrointestinal Risk Factors and Non-steroidal Anti-inflammatory Drugs Use in Rheumatoid Arthritis and Osteoarthritis Patients in Korea. ***J Rheum Dis*** 2016; 23(1):47-54. doi:10.4078/jrd.2016.23.1.47

15. Herrmann M, Anders S, Straub RH, Jenei-Lanzl Z. TNF inhibits catecholamine production from induced sympathetic neuron-like cells in rheumatoid arthritis and osteoarthritis in vitro. ***Sci Rep*** 2018; 8(1):9645. doi:10.1038/s41598-018-27927-8.

16. Saad S, Rashad N. The Relation between *Helicobacter pylori* Infection and the Severity of Rheumatoid Arthritis. ***Bahrain Med Bull*** 2014; 36(4):214-16. doi:10.12816/0008126.

17. Kim HJ, Kim Y-J, Lee HJ, Hong JY, Park AY, Chung EH, *et al*. Systematic review and meta-analysis: Effect of *Helicobacter pylori* eradication on chronic spontaneous urticaria. ***Helicobacter*** 2019; 24(6):e12661. doi:10.1111/hel.12661.

18. Burgos R, Ordoñez G, Vázquez-Mellado J, Pineda B, Sotelo J. Occasional presence of herpes viruses in synovial fluid and blood from patients with rheumatoid arthritis and axial spondyloarthritis. ***Clin Rheumatol*** 2015; 34(10):1681-86. doi:10.1007/s10067-015-2974-2.

19. Larionova RV, Arleevskaya MI, Kravtsova OA, Validov S, Renaudineau Y. In seroconverted rheumatoid arthritis patients a multi-reactive anti-herpes IgM profile is associated with disease activity. ***Clin Immunol*** 2019; 200:19-23. doi:10.1016/j.clim.2019.01.004.

20. Smyk DS, Koutsoumpas AL, Mytilinaiou MG, Rigopoulou EI, Sakkas LI, Bogdanos DP. *Helicobacter pylori* and autoimmune disease:cause or bystander. ***World J Gastroenterol*** 2014; 20(3):613-29. doi:10.3748/wjg.v20.i3.613.

21. Magen E, Delgado J-S. *Helicobacter pylori* and skin autoimmune diseases. ***World J Gastroenterol*** 2014; 20(6):1510-16. doi:10.3748/wjg.v20.i6.1510.

22. Kandil ME, El Hamshary A, Emara NA. Seroprevalence of *Helicobacter pylori* in juvenile Rheumatoid arthritis and its relation to disease severity. ***J Med Sci*** 2007; 7(5):716-23. doi:10.3923/jms.2007.716.723

23. Ebrahimi A, Soofizadeh B, Ebrahimi F, Moaadab SY, Bonyadi M, Gojazadeh M. *et al*. Relationship between *Helicobacter pylori* cytotoxin-associated gene A protein with clinical outcomes in patients with rheumatoid arthritis. ***Immunol Lett*** 2019; 211:49-52. doi:10.1016/j.imlet.2019.05.014.

24. Jones STM, Clague RB, Eldridge J, Jones DM. serological evidence of infection with *Helicobacter pylori* may predict gastrointestinal intolerance to non-steroidal anti-inflammatory drug (nsaid) treatment in rheumatoid arthritis. ***Br J Rheumatol*** 1991; 30(1):16-20. doi:10.1093/rheumatology/30.1.16.

25. Zentilin P, Seriolo B, Dulbecco P, Caratto E, Iiritano E, Fasciolo D. *et al*. Eradication of *Helicobacter pylori* may reduce disease severity in rheumatoid arthritis. ***Aliment Pharmacol Ther*** 2002; 16(7):1291-99. doi:10.1046/j.1365-2036.2002.01284.x